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Research Article

Chemical composition and *in-vitro* anti-arthritic activity of essential oils extracted from the leaves of *Lagerstroemia speciosa*

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ABSTRACT

The study was designed to determine the chemical composition, and *in-vitro* anti-arthritic activity of the essential oils from the fresh and dry leaves of *Lagerstroemia speciosa* (*L.speciosa*). The essential oils from the fresh and dry leaves of *L.speciosa* were obtained by hydro-distillation technique using Clevenger's apparatus. The chemical composition of the essential oils were analysed by gas chromatography coupled with mass spectrophotometry (GC-MS). Mass spectra were searched against mass spectrometry databases. The anti-arthritic activity was screened by protein denaturation method. The GC-MS analysis of essential oils revealed that totally 38 and 35 chemical components were recognized for fresh and dry leaves respectively that have been identified as Hentriacontane, (4,6,8-Tri-ter-butyl-9-oxa-tricyclo deca-2,4,6-trien-1-yl)-methanol, were found to be major chemical components in fresh and dry leaves respectively. In addition, the results showed the significant *in vitro* anti-arthritic activity of the various concentrations of 50, 100, 200, 400 and 800 µg /ml of essential oils using diclofenac as the standard. From the results, it clearly indicates that the essential oils of leaves of *L. speciosa* possess the higher proportions of long chain hydrocarbon which is responsible for the anti-arthritic activity.

Keywords: Essential oils; Leaves, GC-MS analysis; Anti-arthritic activity; *L. speciosa*

INTRODUCTION

Essential oils extracted from a wide variety of plants and herbs which have been traditionally used in the manufacturing of pharmaceuticals, cosmetics, cleaning products, fragrances, food stuffs, soft drinks, distilled alcoholic beverages, herbicides and insecticides (Aydin C *et al*., 2007).A considerable number of studies have been devoted to the extraction of essential oils from various parts of plants like, leaves, seeds, berries, stem, flowers and roots (Berka-Zougali B *et* al., 2010). The amount of essential oil found in these plants may vary from 0.01 to 10 % based on the parts, area, climate, season, geography condition (Chalchat JC *et al*., 1998; Jerkovic I *et al*., 2002; Boelens M H *et al*., 1992). The consumption of essential oils and perfumes are increasing every year, this leads to be a key factor for oil yields and quality of essential oil. Since ancient times the essential oils are used popularly in aromatherapy, a branch of alternative medicine that claims to the various biological properties like antioxidant, anti-inflammatory, antibacterial, antiviral, stimulates the central nervous system, skin infections, spasmolytic, anti-hydrotic etc (Cragg GM *et al*.,1997; Recio MC., 1989; Faky FK *et al*.,1995).

For example: Lavender extract is used as antiseptic and anti-inflammatory for skin care; menthol is derived from mint and is used in inhalers, pills or ointments to treat nasal congestion; thymol, the major component of thyme essential oil is known for its antimicrobial activity; limonene and eucalyptol appear to be specifically involved in protecting the lung tissue. Therefore, essential oils have become a target for the recovery of natural bioactive substances (Mimica-Dukic N *et al*., 2004; Tuberoso CIG *et al*., 2005).

Essential oils are composed of lipophilic substances, possesses the volatile aroma components of the vegetal matter, which are also involved in the defence mechanisms of the plants. The essential oil represent a small fraction of plant composition, and is comprised mainly by monoterpenes and sesquiterpenes, and their oxygenated derivatives such as alcohols, aldehydes, ketones, acids, phenols, ethers, and esters. The amount of a particular substance in the essential oil composition varies from really high proportions. Nevertheless, components present in traces are also important, since all of them are responsible for the characteristic natural odour and flavour (Anitescu C *et al*., 1997) Thus, it is important that the extraction procedure applied to recover essential oils from plant matrix can maintain the natural proportion of its original components.

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The newer technological approaches gaining some interest in extraction and isolation of these essential oils from raw materials is the recent upcoming research and development. The traditional methods of recovering the essential oils include steam and hydro-distillation, and liquid-solvent extraction, cold pressing, cohobation, maceration and effleurage. Using of appropriate solvents is other best method to extract essential oils (Guan W *et al*., 2007; Liu F *et al*., 2001). *Lagerstroemia speciosa* is commonly known as Banaba belongs to lythraceae family. *Lagerstroemia* is rich sources of secondary metabolites including polyphenols, flavonoids, saponins, alkaloids, glycosides, phytosterols, tannins, and terpenoids, etc (Vijaykumar K *et al*., 2006; Stohs SJ *et al*., 2012; Niranjan MH *et al*., 2010).

Rheumatoid arthritis is an autoimmune disease in which there is joint inflammation, synovial proliferation and destruction of articular cartilage (Singh M *et al*., 2011). Inflammation is a bodily response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed physiological functions. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents (Chandra S *et al*., 2012). The commonly used drug for management for arthritis is Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), which have several adverse effects especially gastric irritation leading to gastric ulcers (Pandey S *et al*., 2010; Tripathi KD., 2008).

To the best of our knowledge, there has been no published detailed information about the composition and anti-arthritic activity of the essential oil of the leaves of *L. speciosa*.

MATERIALS AND METHODS

Source material

The leaves of *L. speciosa* were collected during May to August 2014 from the Garden of VIT University. It was authenticated by Dr. P. Jayaraman, PARC (Plant Anatomy Research Centre), Chennai.

Isolation of the essential oils

Essential oils were obtained from the fresh and dry plant material by hydro-distillation method employing Clevenger- type apparatus. The extraction was carried out for a 4-5 hours. To improve their recovery, the essential oil was extracted with Hexane (Merck), dried over anhydrous sodium sulphate (Merck), until the last traces of water removing and stored in a dark sealed glass bottle at 4 °C. This extract was further taken for analysis of sample. The essential oil yield of fresh and dry leaves was found to be 1.6 and 1.2% (w/v) respectively.

Analysis of the essential oils by GC-MS

The essential oils analysis was performed using a GC Perkin Elmer Turbo Mass Gold MS-auto system coupled to mass detector and employing HP-5 column (30.0m length, 250µm ID, 0.25 µm stationary phase thickness).

An electron ionization system, with ionization energy of 70eV was used for GC-MS detection. Helium (99.99%) was the carrier gas, at a flow rate of 0.8 ml/min with linear velocity of 29.6 cm/s. The gas chromatography conditions were set as the follows: column temperature, initial 60°C for 2 min, ramp-10°C/min to 300°C, hold 6 min. 200 to 250°C at 10°C/min. The mass spectrometry operating parameters were 70eV; ion source temperature 240°C, solvent delay- 2 min., 5 min; scan speed, 2000 amu/s; scan range, 50-600 Da.

Identification of chemical compounds

The components of essential oils were identified based on the comparison of their relative retention times and mass spectra with literature data includes; NIST- National Institute of standards and Technology, mass spectral library of the GC-MS system and quantitative data were obtained electronically from flame ionization detection area percent data (Ebrahimabadi AH *et al*., 2010).

Anti-arthritic activity

Anti-arthritic activity of the essential oils was studied using inhibition of albumin denaturation method. 5 ml of reaction mixture was taken which consists of 0.2 ml of egg albumin, 2.8ml of phosphate buffered saline (pH-6.4) and 2 ml of various concentrations of essential oils (50,100,200,400, and 800µg/ml) were used. Buffer and albumin were served as control. Reaction mixture was incubated at 37 °C for 15min and then at 70 °C for 5 min. Reaction mixture was cooled at room temperature and absorbance was measured at 660 nm using UV-Visible Spectrophotometer (HITACHI). As the reference drug diclofenac sodium was used (Shinde, UA 1999).

The percentage inhibition of different concentration of essential oils and standard drug were calculated by using the following formula,

$$\% \text{ inhibition} = (A_0 - A_1 / A_0) \times 100$$

Where; A_0 was the absorbance of control, and A_1 was the absorbance of test or standard.

Statistical analysis

All data were reported as mean ± SD of triplicates. Data were analysed by an ANOVA. $P \leq 0.001$ was considered as significant level.

RESULTS AND DISCUSSION

The essential oils were extracted from the fresh and dry leaves of *Lagerstroemia speciosa* by hydro-distillation using Clevenger's apparatus. The chemical components of essential oils was analysed by GC-MS (Figure 1 & 2).

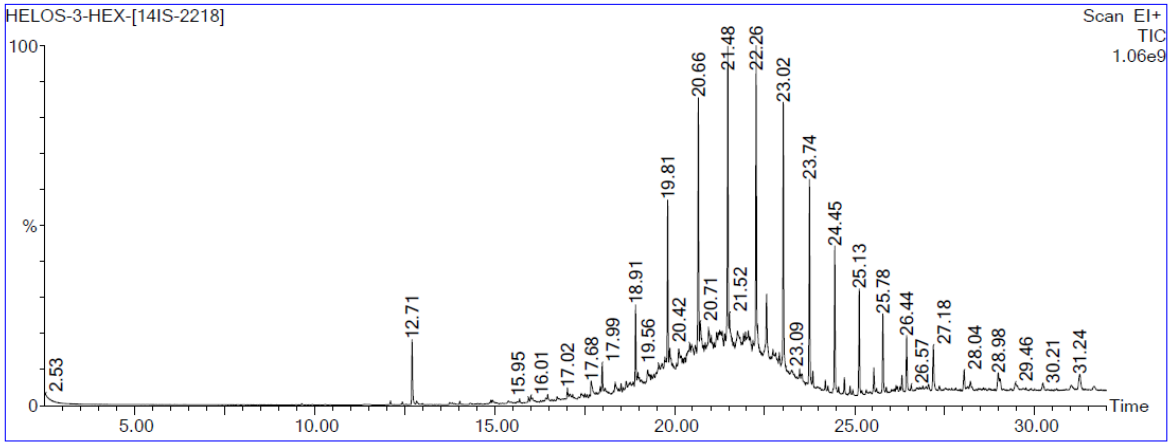

Figure 1: GC-MS of the fresh leaves

Table 1: Major components in the fresh leaves of L. speciosa

RT	Compound name	Molecular formula	Molecular weight	Area %
22.256	Hentriacontane	$C_{31}H_{64}$	436	9.920
27.183	Di-N-Decylsulfone	$C_{20}H_{42}SO_2$	346	2.180
29.024	Trimethyl[4-(1,1,3,3,-tetramethylbutyl)phenoxy]silane	$C_{17}H_{30}SiO$	278	0.965
31.250	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	$C_{16}H$	578	1.325

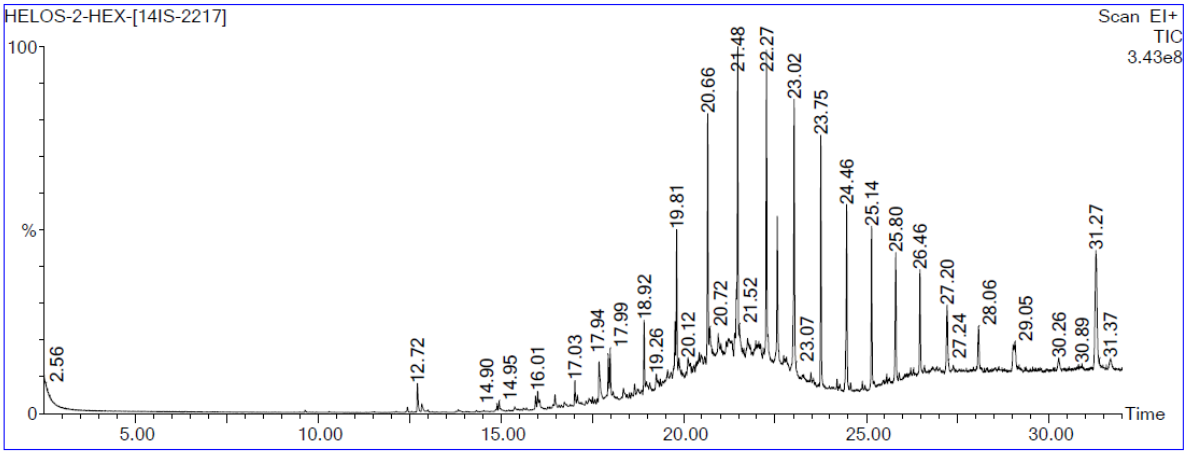

Figure 2: GC-MS of the dry leaves

Table 2: Major components in the dry leaves of L. speciosa

RT	Compound name	Molecular for-mula	Molecular weight	Area%
17.970	Hentriacontane	$C_{31}H_{64}$	436	1.089
25.163	Silane, trichlorooctadecyl	$C_{18}H_{37}Cl_3Si$	386	4.197
29.029	Cyclotrisiloxane, Hexamethyl	$C_6H_{18}Si_3O_3$	222	1.35
31.290	(4,6,8-Tri-ter-butyl-9-oxa-tricyclo deca-2,4,6-trien-1-yl)-methanol	$C_{23}H_{36}O_2$	344	7.330

Totally 38 and 35 different chemical components were identified in fresh and dry leaves respectively. Few of the major compounds were tabulated in Table 1 & 2. The long chain hydrocarbons like hentriacontane was found to be predominant in fresh leaves, while in dry leaves, (4,6,8-Tri-ter-butyl-9-oxa-tricyclo deca-2,4,6-trien-1-yl)-methanol were found to be predominant in dry leaves.

Anti-arthritic activity

Anti-arthritic activity of essential oils extracted from the fresh and dry leaves of *Lagerstroemia speciosa* was evaluated by inhibition of protein denaturation method and values were compared with Diclofenac Sodium, a standard drug used for arthritis pain. *Lagerstroemia*

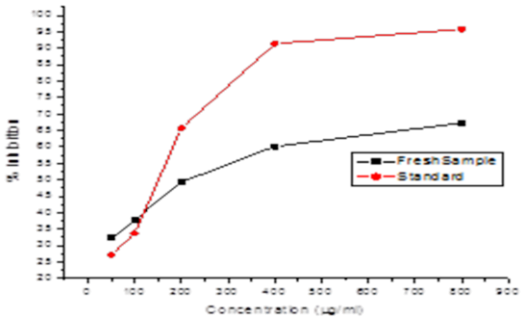

Figure 3: Anti-arthritic activity of fresh leaves

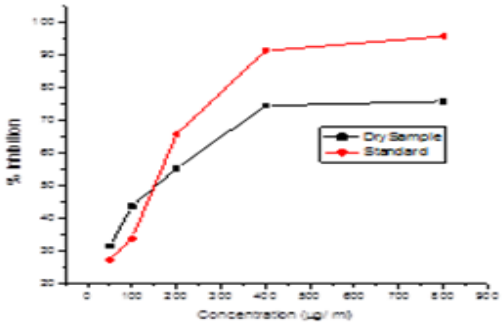

Figure 4: Anti-arthritic activity of dry leaves

speciosa showed inhibition of protein denaturation as shown in Figure 3 & 4.

Denaturation of tissue proteins is one of the well documented causes of inflammatory and arthritic diseases. Production of auto-antigen in certain arthritic diseases results in denaturation of protein and amino acids *in-vivo*. (Brown JH *et al*., 1968) The mechanism of denaturation involves alteration in electrostatic, hydrogen bond, hydrophobic and disulphide bonding. From the present study it can be stated that *L. speciosa* is capable of controlling the production of auto-antigen due to *in-vivo* denaturation of proteins in rheumatic diseases (Umapathy E *et al*., 2010; Deshpande V *et al*., 2009). Moreover, the increasing scientific evidence which links essential oils components with favourable effects on human diseases permit to predict an increase of the application of technologies to extract and isolate these substances from plant matrix, with the consequent application in the production of functional foods, nutraceuticals and pharmacy products.

CONCLUSION

This present study revealed that the GC-MS analysis of essential oils extracted from the fresh and dry leaves of *L. speciosa* can provide standard fingerprints and can be used as a reference for the identification and quality control of the drug. The essential oils possess the anti-arthritic activity may be due to the presence of chemical components such as Hentriacontane, (4,6,8-Tri-ter-butyl-9-oxa-tricyclo deca-2,4,6-trien-1-yl)-methanol. Further study in an animal model to confirm the anti-arthritic activity of essential oils is warranted.

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